INHIBITION OF *PYTHIUM* SPP. AND SUPPRESSION OF
PYTHIUM BLIGHT AND ANTHRACNOSE WITH
PHOSPHONATE FUNGICIDES

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ABSTRACT

Turfgrass diseases are a limiting factor in the maintenance of turfgrasses. Most diseases can be managed using an integrated program of cultural practices, resistant species and cultivars, and fungicides. One option for the integrated control of Pythium blight \([Pythium aphanidermatum\) (Edson) Fitzp. and other Pythium spp.] and anthracnose diseases \([Colletotrichum cereale\) Mans (Crouch, Clarke and Hillman)] of turfgrasses is the use of phosphonate fungicides. Phosphonate compounds are composed of the salts and esters of phosphorous acid \([HPO(OH)_2]\) and are formulated as fungicides and fertilizers. Phosphonate fungicides suppress Pythium blight and anthracnose diseases when applied preventatively, but efficacy may vary with product, rate and timing of application, and host species.

The objectives of this study were to assess the inhibitory effects of phosphorous acid on Pythium spp. and C. cereale \(in vitro\), and determine if active ingredient and formulation of phosphonate fungicides provide similar levels of Pythium blight suppression on perennial ryegrass \((Lolium perenne\) L.) and creeping bentgrass \((Agrostis stolonifera\) L.) and anthracnose basal rot on an annual bluegrass \((Poa annua\) L.) putting green when applied at equivalent rates of phosphorous acid.

Phosphorous acid EC\(_{50}\) values for \(P. aphanidermatum\) isolates ranged from 35.6 to 171.8 µg ml\(^{-1}\). EC\(_{50}\) values for isolates of six other Pythium spp. were between 38.7 and 220.8 µg ml\(^{-1}\). None of the C. cereale isolates used in the \(in vitro\) study were
inhibited by phosphorous acid concentrations up to 1000 µg ml\(^{-1}\), and growth of two isolates increased slightly with increasing concentrations of phosphorous acid.

In 2004 and 2005, all phosphonate treatments provided significant suppression of Pythium blight symptoms on creeping bentgrass and perennial ryegrass relative to the untreated control. No differences in percentage of blighted turf occurred among phosphonate treatments when applied at equivalent rates of phosphorous acid in either year of the study, regardless of active ingredient, formulation, or turfgrass species.

Of the phosphonate treatments included in the anthracnose study, fosetyl Al formulated with a blue-green pigment provided the most consistent and highest degree of anthracnose symptom suppression over two growing seasons. The fact that fosetyl Al/pigment suppressed anthracnose symptoms and a formulation of fosetyl Al without the pigment did not when applied at the same rate of active ingredient, indicates that formulation differences may account for the improved anthracnose control. The only other phosphonate treatment that provided suppression of anthracnose was a non-commercial potassium phosphite solution with no formulation enhancements, but this only occurred in 2005 and was less effective than fosetyl Al/pigment. All phosphonate treatments improved turfgrass quality relative to the untreated control, but fosetyl Al/pigment showed the highest quality ratings over both growing seasons.
# TABLE OF CONTENTS

LIST OF FIGURES........................................................................................................ vii

LIST OF TABLES........................................................................................................ viii

ACKNOWLEDGEMENTS.............................................................................................. ix

Chapter 1. LITERATURE REVIEW............................................................................... 1
    Chemistry of Phosphonate Compounds................................................................. 2
    Phosphonates as Fungicides.................................................................................. 4
        Mode of action.................................................................................................. 7
        Resistance risk................................................................................................. 9
    Phosphonates as Fertilizers.................................................................................. 10
        Effects on turfgrass quality............................................................................. 13
        Phosphonates and the phosphate starvation response................................. 15

Chapter 2. INHIBITION OF *PYTHIUM* SPP. AND SUPPRESSION OF
PYTHIUM BLIGHT WITH PHOSPHONATE FUNGICIDES......................................17
    Introduction.......................................................................................................... 17
    Materials and Methods......................................................................................... 19
        *Pythium* isolates.......................................................................................... 19
        *In vitro* experiments with phosphorous acid, phosphoric acid, and mefenoxam.................................................................................................................. 20
Suppression of Pythium blight with potassium phosphite, fosetyl Al, and mefenoxam
Results and Discussion

In vitro experiments with phosphorous acid, phosphoric acid, and mefenoxam
Suppression of Pythium blight symptoms with potassium phosphite, fosetyl Al, and mefenoxam

Chapter 3. SUPPRESSION OF ANTHRACNOSE BASAL ROT AND IMPROVED PUTTING GREEN QUALITY WITH PHOSPHONATE FUNGICIDES

Introduction
Materials and Methods
Isolation, identification, and maintenance of C. cereale isolates
Effect of phosphorous acid and phosphoric acid on C. cereale in vitro

Anthracnose basal rot symptom suppression and turfgrass quality

Results and Discussion

Effect of phosphorous acid and phosphoric acid on C. cereale in vitro

Anthracnose basal rot symptom suppression and turfgrass quality

LITERATURE CITED
LIST OF FIGURES

1. Effect of phosphonate fungicides on Pythium blight development of cv. Penncross creeping bentgrass in 2004 ................................................................. 33

2. Effect of phosphonate fungicides on Pythium blight development on cv. Integra perennial ryegrass in 2004 ................................................................. 34

3. Effect of phosphonate fungicides on Pythium blight development of cv. Penncross creeping bentgrass in 2005 ................................................................. 35

4. Effect of phosphonate fungicides on Pythium blight development on cv. Integra perennial ryegrass in 2005 ................................................................. 36
LIST OF TABLES

1. Species, isolate, isolate origin, collection date, and host of Pythium isolates used in in vitro or field studies

2. Phosphorous acid EC\textsubscript{50} values for seven species and 24 isolates of Pythium, and mefenoxam EC\textsubscript{50} values for seven \textit{P. aphanidermatum} isolates

3. Analysis of variance for percentage of blighted turf as influenced by treatments and turfgrass species

4. Treatments, rates, and anthracnose basal rot disease severity ratings for 2004 and 2005 phosphonate fungicide tests

5. Treatments, rates, and quality ratings for the 2004 phosphonate fungicide test

6. Treatments, rates, and quality ratings for the 2005 phosphonate fungicide test
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Chapter 1

LITERATURE REVIEW

Phosphonate fungicides are composed of the salts or esters of phosphorous acid \([\text{HPO(OH)}_2]\) or \((\text{H}_3\text{PO}_3)\), and most have potassium phosphite (mono- and di-potassium salts of phosphorous acid) or fosetyl Al [aluminum tris (O-ethyl phosphonate)] as active ingredients (Guest and Grant, 1991). Phosphonate fungicides are used by golf course managers to control Pythium diseases, suppress anthracnose basal rot, and improve turf quality and rooting. In many areas of the United States, phosphonate products are applied at regular intervals throughout the summer as part of a fairway and putting green management program.

Although much is known of phosphonate fungicide efficacy on diseases of nut, fruit, and ornamental crops caused by \textit{Phytophthora} spp., less is understood about their influence on turfgrasses and turfgrass diseases. This literature review will focus on the role of phosphonate compounds as fungicides and fertilizers, and their effects on turfgrasses and turfgrass diseases. At least three comprehensive reviews of phosphonate compounds have been published in recent years, and the reader can refer to these for more detail on specific topics (Cohen and Coffey, 1986; Guest and Grant, 1991; McDonald et al., 2001).
Chemistry of Phosphonate Compounds

In the broadest sense, the term phosphonate describes any compound containing a carbon to phosphorus bond. Some examples of phosphonate compounds include organophosphate insecticides, antiviral medicines, flame retardants, and some herbicides. Phosphonate compounds also occur naturally in some lower life forms, including protozoa, mollusks, coelenterates, and certain fungi (Guest and Grant, 1991).

For this literature review, the term phosphonate is used to describe only those products made from the salts and esters of phosphorous acid. Phosphorous acid is a solid substance available from various chemical supply companies. It is prepared by the controlled hydrolysis of phosphorus trichloride in ice-cold water (Guest and Grant, 1991). When mixed with water, phosphorous acid forms a strong acid called phosphonic acid (Guest and Grant, 1991; Rickard, 2000). This acid is too strong to be used on plants and must be combined with other chemicals to raise the pH of the solution and decrease the potential for plant injury (Ouimette and Coffey, 1989).

One means of reducing the acidity of phosphonic acid is to neutralize it with an alkali salt, typically potassium hydroxide (KOH) (Ouimette and Coffey, 1990). The resulting solution contains mono- and di-potassium salts of phosphorous acid, and is often referred to as potassium phosphite. Potassium phosphite is the active ingredient in several commercial phosphonate fungicides, including Alude, Magellan, Vital, and Resyst (Anonymous, 2005). Potassium phosphite is also the main ingredient in numerous phosphonate fertilizer products.
Phosphonic acid can also be reacted with ethanol to form ethyl-phosphonate. Aluminum ions are added during the manufacturing process to neutralize the ethyl-phosphonate ions, and the resulting product is referred to as fosetyl Al or aluminum tris (O-ethyl phosphonate) (Guest and Grant, 1991; McDonald et al., 2001). This is the active ingredient in Aliette and Chipco Signature fungicides, now marketed by Bayer Environmental Science (Anonymous, 2005). Following uptake by plants, ethyl phosphonate is rapidly hydrolyzed to phosphorous acid, and then to the phosphite anion (H$_2$PO$_3^-$ or HPO$_3^{2-}$) (McDonald et al., 2001; Ouimette and Coffey, 1990).

Phosphonate fungicides and fertilizers should not be confused with phosphate-derived fertilizers such as ammonium phosphate and triple super phosphate. Even though phosphonate and phosphate compounds are very similar chemically, they differ significantly in how they act in plants and fungi.

Phosphate (H$_2$PO$_4^-$ or HPO$_4^{2-}$) is taken up by plants and incorporated into cells where it forms an important energy-yielding molecule (adenosine triphosphate or ATP) and structural components of cell membranes and DNA (McDonald et al., 2001). It is essential for root growth, photosynthesis, and respiration in plants. Thus, it is the primary source of phosphorus in most turfgrass fertilizers. Phosphate does not have a strong direct effect on turfgrass diseases, although phosphorus-deficient plants can be more susceptible to certain diseases than phosphorus-sufficient plants (McDonald et al., 2001).

The phosphite ion differs from the phosphate ion in that a hydrogen atom replaces an oxygen atom at one of the four points of the tetrahedral molecular structure (McDonald et al., 2001). This substitution results in an asymmetrical arrangement of the phosphite ion (phosphate ions are symmetrical). Many of the important enzymes which
react with phosphate and catalyze vital biochemical reactions in plants do not bind in the same manner to the phosphate ion. Thus, phosphite cannot substitute for phosphate in important biochemical reactions such as photosynthesis, respiration, or in the formation of ATP, nucleic acid, and phospholipids (McDonald et al., 2001).

Phosphonate compounds are absorbed by plants and incorporated into cells as phosphite ions (Ouimette and Coffey, 1990). Although phosphite ions can be transported into plant cells, they do not appear to be involved in normal avenues of phosphorus metabolism. Phosphite ions have direct fungitoxic effects on certain plant pathogens, a benefit that is not found with phosphate ions (Fenn and Coffey, 1984).

**Phosphonates as Fungicides**

Fungicidal properties of phosphonates were discovered by scientists at Rhone-Poulenc Agrochemical Laboratories in France during the 1970s (Guest and Grant, 1991). These scientists were screening various chemicals for fungicidal properties when they discovered that phosphonate salts were effective in controlling diseases caused by a group of Oomycete fungi in the Peronosporales order (*Phytophthora*, *Plasmopara*, *Pythium* and others). Soon after this discovery, fosetyl Al was formulated under the trade name Aliette and released for commercial use (Cohen and Coffey, 1986; Guest and Grant, 1991).

Researchers in the early 1980’s reported good control of various Phytophthora diseases of avocado, citrus, pineapple, and ornamental plants with fosetyl Al (Allen et al., 1980; Davis, 1982; Munneckke, 1982). Fosetyl Al was also found to be effective in
controlling Pythium blight of golf course turf and was used primarily on greens and fairways as a preventive treatment (Sanders et al., 1983). Sanders et al. (1983) stated that fosetyl Al showed no toxicity to 25 isolates representing eight *Pythium* spp. *in vitro*, but adequately suppressed Pythium blight symptoms in the field. The authors concluded that field control of Pythium blight with fosetyl Al may result from elicitation of antifungal responses in the host.

In the early 1990s, Lucas (1995) found that fosetyl Al combined with a commercial mancozeb fungicide (Fore) improved turf quality and controlled what was referred to as “summer decline of bentgrass” or “summer stress complex”. Based on this discovery, Rhone-Poulenc scientists developed and patented a formulation containing fosetyl Al and a blue-green pigment contained in Fore, which produced results similar to the Aliette/Fore combination (Mudge, 1997). Based on this finding, a product called Chipco Signature was developed and commercialized. Chipco Signature has since become widely used on golf courses throughout the United States. Chipco Signature and Aliette are labeled for control of Pythium diseases and yellow tuft in turf. They are also labeled for control of summer stress complex when combined with one of several other fungicides (Anonymous, 2005). Chipco Signature is also labeled for the control of anthracnose and bentgrass dead spot diseases when combined with one of several fungicides listed on the label (Anonymous, 2005).

During the mid-1990s, potassium phosphite products entered the turfgrass market and gained popularity as fungicides and fertilizers. Several of these products have been registered through the Environmental Protection Agency as fungicides (Alude, Magellan, Vital, and Resyst) and have specific information on labels for the control of Pythium
diseases, and in some cases, summer stress complex when combined with a mancozeb fungicide (Anonymous, 2005).

Whereas most turfgrass fungicides are contacts or acropetal penetrants (translocated in plant xylem), phosphonate fungicides possess symplastic ambimobility, or movement in both xylem and phloem (Cohen and Coffey, 1986; Ouimette and Coffey, 1990). Translocation in phloem allows phosphonate fungicides to move from leaf tissues to crowns and roots of plants. Because of this unique property, phosphonates are viewed as excellent fungicides for controlling root diseases such as Pythium root rot and dysfunction caused by various Pythium spp. (Griffith et al., 2000; Jackson et al., 2007).

Phosphonate fungicides have good efficacy for Pythium diseases and other diseases caused by Peronosporales fungi when applied preventively, but they are thought to have poor efficacy when applied post-infection (after disease symptoms and signs are apparent) (Jackson et al., 2000). These fungicides also suppress or control some diseases caused by pathogens unrelated to the Peronosporales, including nematode disorders caused by Heterodera avenae Woll. and Meloidogyne marylandi Jepson and Golden (Oka et al., 2007), bacterial wilt caused by Ralstonia solanacearum (Smith) Yabuuchi et al. (Norman et al., 2006), moldy-core decay caused by Alternaria alternate (Fr.) Keissl. (Reuveni et al., 2003), and anthracnose basal rot caused by Colletotrichum cereale Manns (Crouch, Clarke and Hillman) (Burpee, 2005; Towers et al., 2003; Tredway, 2006). Phosphonate fungicides are not particularly effective in controlling anthracnose basal rot alone, and are combined with other fungicides that have a stronger fungitoxic affect on the pathogen (Burpee, 2005; Wong et al., 2007).
Mode of action

The mode of action of phosphonate fungicides has long been a source of debate and conjecture. Some scientists believe that the primary fungicidal effects of these products are directly on the fungal pathogen (Fenn and Coffey, 1985), whereas others suspect that both a direct effect on the fungus and a stimulation of natural host defenses combine to prevent disease (Smillie et al., 1989).

Early studies with phosphonate fungicides incorporated into artificial growth media showed no direct effect on *Pythium aphanidermatum* (Edson) Fitzp. and other *Pythium* spp. when amended into potato dextrose agar (PDA) with fosetyl Al at 1, 10, and 100 µg ml\(^{-1}\) (Sanders et al., 1983). However, subsequent studies showed that phosphonate fungicide-amended media used by Sanders et al. (1983) did not inhibit fungi because the fungicide concentrations were not high enough. Fenn and Coffey (1984) demonstrated that mycelia of four *Pythium* spp., including *P. aphanidermatum*, were inhibited when phosphorous acid was amended into corn meal agar (CMA) at concentrations of 276 and 552 µg ml\(^{-1}\). Generally, *Phytophthora* spp. are more sensitive to phosphonate compounds than *Pythium* spp., with EC\(_{50}\) values ranging from 2.5 to 224.4 µg ml\(^{-1}\) for nine different *Phytophthora* spp. (Cohen and Coffey, 1986). These EC\(_{50}\) values are considered high compared to EC\(_{50}\) values of other Peronosporales fungicides such as metalaxyl (EC\(_{50}\) = 0.5 to 2.6 µg ml\(^{-1}\) for sensitive *P. aphanidermatum* isolates) (Sanders et al., 1990).

Another reason for poor *in vitro* activity of phosphonate compounds is due to high phosphate concentrations in PDA and other media (Fenn and Coffey, 1984). Phosphite and phosphate compete for the same transporters across cell membranes, and phosphate
tends to out-compete phosphite for access to these sites, thereby blocking uptake of phosphite by fungi (McDonald et al., 2001). Lowering the amount of phosphate in growth media allows the phosphite ion to directly inhibit fungi.

Within the past two decades, our understanding of how phosphonate fungicides affect fungal growth and metabolism has improved. In a study using three *Phytophthora* species, Australian scientists found that phosphonate fungicides interfere with phosphate metabolism by causing an accumulation of two compounds, polyphosphate and pyrophosphate, in fungal cells. Accumulation of these compounds is thought to divert ATP from other metabolic pathways, resulting in a decrease in fungal growth (Niere et al., 1994). More recently, phosphonate fungicides were found to inhibit several key enzymes needed for growth and development in *Phytophthora palmivora* (Butler) Butler (Stehmann and Grant, 2000). These studies suggest that the mode of action of phosphonate fungicides is at least partially due to direct inhibition of the pathogen.

Because of the relatively low toxicity of phosphonate fungicides reported for Peronosporales fungi, some scientists believe the mode of action of phosphonate fungicides is not solely due to pathogen inhibition, but also involves stimulation of the plant’s natural chemical and physical defenses against disease (Grant and Guest, 1991; Smillie et al., 1989; Vo-Thi-Hi et al., 1979). Smillie et al. (1989) reviewed studies focusing on accelerated increases in phytoalexins in response to phosphonate treatment and suggested this could be taken as evidence for a mode of phosphite action in certain plants. Afek and Sztejnberg (1989) reported that scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora* (Smith and Smith) Leonian, was two to four times greater in inoculated branches treated with fosetyl Al or phosphorous
acid than in inoculated, untreated branches. However, when branches were not inoculated, these fungicides had no influence on scoparone concentration, indicating that both the pathogen and the fungicide are involved in initiating the defense response in citrus.

A more recent study involving *Eucalyptus* showed that the concentration of phosphite ions in plants may determine the extent of host defense chemical activation. Jackson et al. (2000) found when concentrations of phosphite ions in the roots of *Eucalyptus* were low, host-defense enzymes (4-coumarate coenzyme A ligase and cinnamyl alcohol dehydrogenase) were stimulated. However, when concentrations of phosphite ions were high, host-defense enzymes remained unchanged, and the phosphite ions inhibited growth of the pathogen before it caused disease (Jackson et al., 2000).

Studies on stimulation of host-defense mechanisms are difficult to conduct and require the ability to detect minute quantities of complex compounds in plants. Therefore, much less is known about this mode of action than the direct fungitoxic effects of phosphonate fungicides. As of this writing, no detailed studies have been published concerning activation of host defenses in phosphonate-treated turfgrasses.

**Resistance risk**

The widespread use of phosphonate products as disease control agents and fertilizers, and for the improvement of turf quality during periods of environmental stress, has led to concerns about the development of pathogen resistance (Vincelli, 2004). To date, no reports of pathogen resistance to phosphonate fungicides in turfgrass have been
published, although phosphonate-resistant mutants of *P. aphanidermatum* have been induced in a laboratory (Sanders et al., 1990).

Two factors may be responsible for the reduced resistance risk with phosphonate products; the mode of action in target fungi may involve multiple sites, and host defense mechanisms induced by phosphorous acid may play a significant role in disease suppression. Both of these factors create a broad front against disease development and a difficult hurdle for pathogens to overcome through resistance. Nevertheless, a recent report from California suggests that sensitivity to phosphonate fungicides was compromised in populations of *Bremia lactucae* Regel (causal agent of lettuce downy mildew) treated repeatedly with phosphonate fungicides and fertilizers (Brown et al., 2004). The California experience may be an isolated case, but it demonstrates that the development of resistance is a possibility with phosphonate fungicides, and that indiscriminant use of these products may lead to problems in the future.

**Phosphonates as Fertilizers**

Phosphonate compounds were first investigated as fertilizers in the United States by scientists from the Tennessee Agricultural Experiment Station (MacIntire et al., 1950). In a preliminary experiment, phosphorous acid and calcium phosphite treatments produced lower yields of red clover (*Trifolium pretense* L.) compared to treatments of phosphate fertilizer. Subsequent experiments involved sequential plantings of different crop species into soils treated with phosphite or phosphate fertilizers. Results revealed that yields of the first crop (domestic ryegrass, *Lolium* spp.) treated with phosphite
sources were lower than those of phosphate-treated ryegrass, and similar to control plants receiving no phosphorus. However, the second crop (soybeans, *Glycine max* (L.) Merr.), which was seeded into the soils treated with phosphite sources, showed higher yields than untreated controls. MacIntire et al. (1950) attributed the higher yield response of the second crop to increasing phosphate concentrations resulting from the oxidation of phosphite to phosphate in the phosphite-treated soils. Unfortunately, intervals between successive crops and initial phosphorus concentrations of the soils used in the experiment were not reported. Also, data listed in the manuscript were not subjected to any type of statistical analysis.

Shortly after MacIntire et al. (1950) published their findings, soil scientists from the University of California examined the mechanism of oxidation of phosphite to phosphate in soils (Adams and Conrad, 1952). The authors determined that oxidation of phosphite to phosphate is mediated by soil microorganisms, and reported that several species of bacteria, actinomycetes, and fungi were able to assimilate phosphite and release phosphate in synthetic culture solutions. They also found that bacteria would not use phosphite until most phosphate in culture solutions was depleted. Adams and Conrad (1952) concluded that the ability to oxidize phosphite to phosphate seems to be characteristic of many types of microorganisms.

In a more recent study, Forster et al. (1998) investigated the growth responses of tomatoes (*Lycopersicon esculentum* L.) to potassium phosphite and potassium phosphate sources in a hydroponic system. The authors reported that plants treated with phosphite sources alone exhibited phosphorus deficiency symptoms similar to the no-phosphorus control plants. Plants treated with potassium phosphate produced greater leaf area, leaf
dry weights, stem dry weights, and root dry weights than phosphite treated plants and the no-phosphorus control plants. Forster et al. (1998) also observed a decrease in the root:shoot ratio of plants treated with potassium phosphite when compared with control plants. The authors attributed the reduced root:shoot ratio to phosphite interfering with the plants’ phosphorus starvation response, a phenomenon previously reported by Carswell et al. (1996). Phosphate concentrations in leaf tissues were higher for phosphate-treated plants than for all phosphite treatments; however, a commercial formulation of potassium phosphite showed higher phosphate concentrations in tomato leaves than leaf tissues from the no-phosphorus control. Forster et al. (1998) concluded that technical and commercial formulations of potassium phosphite did not produce sufficient phosphorus nutrition to tomatoes growing in hydroponic systems.

Limited research on the role of phosphonate compounds as a source of phosphorus fertilizer has been conducted on turfgrasses. Dorer (1996) designed a two-year study to determine the relationship between nutrients supplied by a combination of fosetyl Al and mancozeb (Aliette and Fore fungicides) and quality, disease suppression, and yields of creeping bentgrass (Agrostis stolonifera L.). None of the treatments influenced clipping yields when data were averaged over the 1994 growing season. When applied alone, fosetyl Al and phosphorous acid treatments did not improve mean clipping yields relative to the untreated control in 1995. However, several treatments containing combinations of phosphonate compounds and mancozeb and/or micronutrients improved yields in 1995. No differences in root development were observed among treatments at any time during the experiment. This study provided little data to support claims that significant phosphorus nutrition was supplied by phosphonate compounds.
No phosphate compounds were included in the experiment for comparison with phosphonate treatments.

Although most studies examining the nutritional effects of phosphite fertilizers have shown no improvements in yield (prior to the oxidation of phosphite to phosphate), Rickard (2000) presented data from several non-peer reviewed reports showing higher yields of phosphite-treated vegetable and fruit crops when compared to untreated controls. McDonald et al. (2001) suggested that claims of higher yields in phosphite-treated crops could be due to the conversion of phosphite to phosphate in treated soils, or from the suppression of root-debilitating pathogens in some of the field trails.

Despite research findings cited above, phosphonate compounds are still marketed by some companies as a source of phosphorus and potassium fertilizer. Although phosphite can be converted to phosphate in soil, turfgrass managers should realize that this is an inefficient means of supplying phosphorus to plants when compared with phosphate fertilizer.

**Effects on turfgrass quality**

Although research shows phosphonate compounds do not directly affect yield in grasses, some phosphonate products have demonstrated improved turf quality (darker green color, higher density, uniformity, and freedom from diseases and other pest symptoms), although results are highly variable from year to year and region to region. Tredway (2006) reported significant quality improvement of an annual bluegrass (*Poa annua* L.) putting green with potassium phosphite (Alude) and fosetyl Al (Chipco Signature) in North Carolina during 2005, but little to no improvement on creeping
bentgrass greens in North Carolina during 2003 and 2007 (Tredway and Butler, 2004; Soika and Tredway, 2007a). In 2002, a study conducted on an annual bluegrass green in California showed no quality or color improvement resulting from multiple applications of Chipco Signature at label-prescribed rates; whereas, a trial during the same year in New Jersey showed significant quality enhancement of an annual bluegrass green with the same fungicide applied at the same rate and timing as in the California trial.

Quality enhancement with phosphonate products is probably not due to phosphorus nutritional effects, as previously cited studies have shown no short-term improvement from phosphite fertilizer. However, Dorer (1996) reported turfgrass quality improvements resulting from fosetyl Al/mancozeb (Aliette/Fore) combinations were correlated with several nutrients, including potassium, sulfur, zinc, and manganese. Zinc and manganese are found in mancozeb and not in fosetyl Al, and the Fore formulation contains a blue-green pigment that is known to enhance color and other quality parameters (Vincelli and Dixon, 2005). Also, fosetyl Al has the ability to suppress some plant pathogens, and could have influenced quality through suppression of debilitating root and foliar diseases. Thus, the quality improvement resulting from Aliette/Fore treatments may not have been due to nutritional effects resulting from the fosetyl Al.

Formulation enhancements, as in the case of Chipco Signature fungicide, have in some cases led to turf quality improvement over other phosphonate products (Vincelli and Dixon, 2005; Dernoeden, 2008). Dernoeden (2008) attributed most of the quality improvement of Chipco Signature to a blue-green pigment (currently referred to as StressGard), and stated that cursory studies with this product showed no elevated chlorophyll or nutrient levels in treated turf, and that respiration and canopy temperature
were not affected. Unpublished studies at Virginia Tech indicate that the Chipco Signature formulation sustains high levels of superoxide dismutase in creeping bentgrass for up to 20 days under heat-stress conditions. Superoxide dismutase is an antioxidant enzyme correlated with improved tolerance to heat stress and a delay in leaf senescence in turfgrasses (Erik Ervin, personal communication).

Although it is clear that in some cases phosphonate fungicides improve turfgrass quality, other non-phosphonate fungicides can also improve turf quality (Dernoeden, 2008; Tredway, 2006; Soika and Tredway, 2007a). Quality improvement may simply be due to the suppression of minor, plant-debilitating pathogens, such as Pythium species. More research is needed to determine the precise cause of enhanced turf quality.

**Phosphonates and the phosphate starvation response**

Plants growing in phosphorus-deficient environments undergo metabolic changes which allow survival during extended periods of phosphate starvation. Duff et al. (1989) found that levels of phosphoenolpyruvate (PEP) phosphatase and other phosphate-independent enzymes increase in phosphate-starved Brassica nigra L. to provide a means of bypassing the ADP-dependent pyruvate kinase reaction (a glycolytic reaction used in respiration) when intracellular pools of ATP, ADP, and phosphate are depleted.

Carswell et al. (1996) found that growth of phosphate-starved B. nigra seedlings was suppressed when low concentrations (1-2 mM) of phosphite were added to growth media. When phosphate-starved plants were treated with phosphite, intracellular levels of phosphate decreased, and phosphite accumulated in leaves and roots at levels up to 16 times that of phosphate in phosphate-starved plants not treated with phosphite. Phosphite
treatments also reduced the induction of PEP phosphatase and pyrophosphate-dependent phosphofructokinase activities by 40 to 90%, thereby interrupting the regulation of phosphate starvation responses.

In a subsequent study, Carswell et al. (1997) found that phosphite additions to phosphate-starved *Brassica napus* L. cell suspensions largely abolished the phosphate-starvation-dependent induction of pyrophosphate-dependent phosphofructokinase, acid phosphatase, and the high-affinity plasmalemma phosphate transporter. The authors hypothesized that a primary site of phosphite activity in higher plants is at the signal transduction chain by which plants perceive and respond to phosphate stress.

The findings of Carswell and associates help to explain why phosphate-deficient tomato plants treated with phosphite sources developed phosphorus deficiency symptoms and lower root:shoot ratios in the study conducted by Forster et al. (1998). Presumably, a similar response is possible in turfgrass establishments if phosphite-based fertilizer products are substituted for phosphate fertilizer in phosphorus-deficient soils.
Chapter 2

INHIBITION OF *PYTHIUM* SPP. AND SUPPRESSION OF 
*PYTHIUM* BLIGHT WITH PHOSPHONATE FUNGICIDES

Introduction

Pythium blight, caused by *P. aphanidermatum* and other *Pythium* spp., is a destructive foliar disease of turfgrasses in many areas of the United States. In northern climates, this disease occurs on poorly-drained perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass golf course fairways during periods of high temperature and humidity (Nutter et al., 1983; Smiley et al., 2005). Golf course superintendents typically manage Pythium blight using cultural practices and applications of fungicides when conditions favor disease development (Vargas, 1994). Penetrant fungicides that are effective in controlling Pythium blight when applied prior to symptom development include mefenoxam, propamocarb, strobilurins, and the phosphonates (Burpee et al., 2005b; Uddin et al., 2002; Uddin et al., 2003; Vargas, 1994).

Phosphonate fungicides are composed of the esters or salts of phosphorous acid, and most have fosetyl Al or potassium phosphite as active ingredients (Guest and Grant, 1991). Phosphonate fungicides control many diseases caused by * Phytophthora* spp., *Pythium* spp., and some other plant pathogens. Unique properties of these fungicides include significant translocation in xylem and phloem, and a dual mode of action that
involves direct fungitoxic effects and stimulation of certain host defense responses (Guest and Grant, 1991).

Although considerable research has been conducted on the fungitoxic effects of phosphonate fungicides on *Phytophthora* spp., relatively few studies have been carried out on species of *Pythium*. Sanders et al. (1983) found no mycelial inhibition of 25 isolates of *Pythium* representing eight species, on PDA amended with fosetyl Al at 1, 10, or 100 µg ml\(^{-1}\). Fenn and Coffey (1984) reported that mycelia of four *Pythium* species were inhibited when phosphorous acid was amended into CMA at concentrations of 276 and 552 µg ml\(^{-1}\). The authors noted considerable variation in growth responses among the four species at low phosphorous acid concentrations, with *P. aphanidermatum* inhibited and *P. ultimum* Trow stimulated at 69 µg ml\(^{-1}\). In a separate experiment, Fenn and Coffey (1984) found one isolate of *P. aphanidermatum* was more strongly inhibited at 69 µg ml\(^{-1}\) phosphorous acid in the presence of 0.84 mM phosphate compared with 8.4 mM phosphate. Detailed information is needed on the reaction of a range of *Pythium* spp. and isolates to varying concentrations of phosphorous acid *in vitro*.

Field studies comparing phosphonate fungicides for Pythium blight control have been conducted at different locations and on different turfgrass species (Burpee et al., 2005b; Datnoff et al., 2001; Datnoff et al., 2004; Uddin et al., 2002; Uddin et al., 2003; Uddin et al., 2004; Uddin et al., 2005). In most of these studies, both fosetyl Al and potassium phosphite products reduced Pythium blight symptom development; however, in some cases, results varied depending on the product (Uddin et al., 2004; Uddin et al., 2005), product rate (Uddin et al., 2004; Uddin et al., 2005), and application timing (Datnoff et al., 2004; Uddin et al., 2002). In many of these studies, phosphonate products
were not compared at equivalent concentrations of phosphorous acid. Because of potential efficacy and price differences among the active ingredients and formulations of phosphonate fungicides, golf turf managers would benefit from comparisons of phosphonate products applied at the same concentration of phosphorous acid and on different turfgrass species.

The objectives of this study were to assess the inhibitory effects of phosphorous acid on mycelial growth of different species and isolates of *Pythium*, and determine if active ingredient and formulation of phosphonate fungicides provide similar suppression of Pythium blight in the field when applied at equivalent rates of phosphorous acid on perennial ryegrass and creeping bentgrass.

**Materials and Methods**

*Pythium isolates*

*Pythium aphanidermatum* isolates were obtained from a collection maintained in the Dept. of Plant Pathology at The Pennsylvania State University by W. Uddin. Isolates of other *Pythium* spp. (*P. graminicola* Sub., *P. irregular* Buisman, and *P. ultimum*) were supplied by C. Stiles from a study conducted in Florida (Stiles et al., 2005) or from a collection maintained by G. Moorman at The Pennsylvania State University (*P. irregulare, P. ultimum*, and *P. myriotylum* Drechsler). One isolate of *P. volutum* Vanterpool and Truscott and two isolates of *P. torulosum* Coker and Patterson were provided by L. Tredway, North Carolina State University and J. Kerns, University of Wisconsin (formerly of North Carolina State University). *Pythium* spp., isolate
designation, state of origin, year of isolation, and host (if known) for individual isolates are listed in Table 1. All isolates were maintained on slants of PDA (BBL, Becton, Dickson, and Co., Sparks, MD) and covered with autoclaved mineral oil. Isolates were transferred to new PDA slants every 3 months.

**In vitro experiments with phosphorous acid, phosphoric acid, and mefenoxam**

In vitro experiments were conducted to assess the effects of different concentrations of phosphorous acid and phosphoric acid (H₃PO₄) on mycelial growth of 23 isolates of *P. aphanidermatum*, *P. graminicola*, *P. irregulare*, *P. myriotylum*, *P. torulosum*, *P. ultimum*, and *P. volutum*; and of mefenoxam concentrations on seven isolates of *P. aphanidermatum*.

Phosphorous acid solutions were prepared by adjusting a 1 M solution of reagent-grade phosphorous acid (Sigma-Aldrich, St. Louis) to a pH of 6.2 by titrating with 10 M reagent-grade potassium hydroxide (KOH, Sigma-Aldrich) (Coffey and Joseph, 1985). Phosphoric acid solutions were made by adjusting a 1 M solution of reagent-grade phosphoric acid (Sigma-Aldrich) to a pH of 6.2 by titrating with 10 M reagent-grade KOH. These solutions were used to amend CMA (BBL, 17 g L⁻¹) with 10, 50, 100, 500, and 1000 µg ml⁻¹ of phosphorous acid, or equivalent concentrations of phosphoric acid. Phosphoric acid treatments were used as controls to account for the potential inhibitory effects of potassium hydroxide, which was used to adjust the pH of the phosphorous acid. Technical-grade mefenoxam (Syngenta Professional Products, Greensboro, NC) was dissolved in acetone and amended into CMA at 0.01, 0.1, 1, 10, and 100 µg ml⁻¹. Mefenoxam was dissolved in 2 ml acetone per L of medium for each concentration.
Table 1. Species, isolate, isolate origin, collection date, and host of *Pythium* isolates used in *in vitro* or field studies.

<table>
<thead>
<tr>
<th><em>Pythium</em> species</th>
<th>Isolate designation</th>
<th>Origin</th>
<th>Year collected</th>
<th>Host species</th>
</tr>
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<tbody>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>P-3</td>
<td>PA</td>
<td>1974</td>
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<td>PA</td>
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<td>P-27</td>
<td>PA</td>
<td>1976</td>
<td>unknown</td>
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<td>P-32</td>
<td>PA</td>
<td>1986</td>
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<td>P-38</td>
<td>PA</td>
<td>1988</td>
<td>unknown</td>
</tr>
<tr>
<td><em>P. aphanidermatum</em></td>
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<td>PA</td>
<td>1994</td>
<td><em>L. perenne</em></td>
</tr>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>P-41</td>
<td>PA</td>
<td>1991</td>
<td><em>L. perenne</em></td>
</tr>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>Zen 97-71</td>
<td>CA</td>
<td>1997</td>
<td>unknown</td>
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<tr>
<td><em>P. aphanidermatum</em></td>
<td>Zen 97-375</td>
<td>unknown</td>
<td>1998</td>
<td>unknown</td>
</tr>
<tr>
<td><em>P. aphanidermatum</em></td>
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<td>CA</td>
<td>1998</td>
<td>unknown</td>
</tr>
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<td><em>P. ultimum</em></td>
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<td><em>Chrysanthemum</em> sp.</td>
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<td><em>P. ultimum</em></td>
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<td>PA</td>
<td>1998</td>
<td><em>Pelargonium</em> X hortorum</td>
</tr>
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<td><em>P. ultimum</em></td>
<td>P-19</td>
<td>PA</td>
<td>1996</td>
<td><em>Euphorbia pulcherrima</em></td>
</tr>
<tr>
<td><em>P. ultimum</em></td>
<td>G_{5}FB_{3} SL4-14</td>
<td>FL</td>
<td>2002</td>
<td><em>Cynodon</em> spp.</td>
</tr>
<tr>
<td><em>P. ultimum</em></td>
<td>G_{6}FB_{3} SL5-17</td>
<td>FL</td>
<td>2002</td>
<td><em>Cynodon</em> spp.</td>
</tr>
<tr>
<td><em>P. irregulare</em></td>
<td>P-50</td>
<td>PA</td>
<td>1999</td>
<td><em>Euphorbia pulcherrima</em></td>
</tr>
<tr>
<td><em>P. irregulare</em></td>
<td>72076-96</td>
<td>PA</td>
<td>1996</td>
<td><em>Euphorbia pulcherrima</em></td>
</tr>
<tr>
<td><em>P. irregulare</em></td>
<td>42130-97</td>
<td>PA</td>
<td>1997</td>
<td><em>Pelargonium</em> X hortorum</td>
</tr>
<tr>
<td><em>P. myriotylum</em></td>
<td>P-49</td>
<td>PA</td>
<td>1999</td>
<td><em>Pelargonium</em> X hortorum</td>
</tr>
<tr>
<td><em>P. myriotylum</em></td>
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<td>PA</td>
<td>1999</td>
<td><em>Pelargonium</em> X hortorum</td>
</tr>
<tr>
<td><em>P. myriotylum</em></td>
<td>P-30</td>
<td>PA</td>
<td>1998</td>
<td><em>Pelargonium</em> X hortorum</td>
</tr>
<tr>
<td><em>P. torulosum</em></td>
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<td>NC</td>
<td>2003</td>
<td><em>A. stolonifera</em></td>
</tr>
<tr>
<td><em>P. torulosum</em></td>
<td>SV3</td>
<td>SC</td>
<td>2004</td>
<td><em>A. stolonifera</em></td>
</tr>
<tr>
<td><em>P. volutum</em></td>
<td>PRD 48</td>
<td>NC</td>
<td>2003</td>
<td><em>A. stolonifera</em></td>
</tr>
<tr>
<td><em>P. graminicola</em></td>
<td>UAA1</td>
<td>FL</td>
<td>2002</td>
<td>unknown</td>
</tr>
</tbody>
</table>
A preliminary test showed that 2 ml acetone per L of CMA did not influence growth of *P. aphanidermatum* when compared with non-amended CMA. Corn meal agar, not amended with phosphorous acid, phosphoric acid, or mefenoxam served as controls.

All *Pythium* isolates selected for the *in vitro* study were maintained on non-amended CMA at 25°C for 30 h prior to transferring. Agar plugs, 7-mm-diameter, were cut from actively growing margins of each colony and transferred to the center of plastic 90-mm-diameter Petri dishes containing CMA amended with phosphorous acid, phosphoric acid, or mefenoxam, as well as the non-amended CMA. The Petri dishes were incubated in the dark at 25°C. Two replicates were used for each treatment, and treatments were arranged in a randomized complete block design. Radial growth was determined by measuring the colony radius at two points on each dish and recording the average value. Radial growth measurements were taken 30 h after transfer for all isolates of *Pythium aphanidermatum*, *P. irregulare*, *P. myriotylum*, and *P. ultimum*; 72 h after transfer for *P. torulosum* isolates; and 96 h after transfer for *P. volutum* and *P. graminicola* isolates. Percent relative growth was calculated as (radial growth on amended CMA/radial growth on non-amended CMA) ×100. The experiment was performed twice, and data from both runs were similar. Data from both runs were pooled for regression analysis.

Phosphorous acid EC$_{50}$ values (effective concentration that reduces mycelial growth by 50%) for all 23 *Pythium* isolates, and mefenoxam EC$_{50}$ values for seven isolates of *P. aphanidermatum*, were obtained by regressing relative growth against the log$_{10}$ of phosphorous acid or mefenoxam concentrations (SAS 9.1; SAS Institute, Cary,
NC). EC<sub>50</sub> values were not determined for phosphoric acid treatments because none of the concentrations inhibited growth of isolates relative to the non-amended CMA control.

**Suppression of Pythium blight with potassium phosphite, fosetyl Al, and mefenoxam**

This field study was conducted at the Joseph Valentine Turfgrass Research Center, University Park, PA during 2004 and 2005. The soil at the test site is a Hagerstown silt loam with a pH of 6.8, 168.1 kg ha<sup>-1</sup> Mehlich-3 P, 0.54 cmol. exchangeable K kg<sup>-1</sup> of soil, and a CEC of 13.4 cmol<sub>c</sub> kg<sup>-1</sup> of soil. The turfgrasses used in this study were perennial ryegrass (cv. Integra) and creeping bentgrass (cv. Penncross). Both species were established on the test site from seed (195.3 kg ha<sup>-1</sup> perennial ryegrass seed or 48.8 kg ha<sup>-1</sup> creeping bentgrass seed) during September, 2003 and again in September, 2004. The turf was mowed at 2.54 cm every other day with a rotary mower, and fertilized twice per year (spring and summer) with 48.8 kg ha<sup>-1</sup> N per application as isobutylidene diurea (IBDU).

Prior to treatment application in 2004 and 2005, a 9.1 by 14.6 m chamber constructed of an aluminum frame and covered with clear polyethylene plastic was placed over the test site. An automatic misting system designed to increase humidity and cool the turf was suspended from the chamber frame. After fungicide treatments were applied, the two open ends of the chamber were sealed with preassembled wooded frames covered with clear polyethylene plastic. Each end was equipped with a hinged window approximately 1.5 m above the ground that could be opened or closed to facilitate heating or cooling. Two electric heaters equipped with fans and thermostats were placed on either side of the chamber to aid in heating when night temperatures dropped below 16°C.
Treatments included a commercial formulation of potassium phosphite (potassium phosphite-C) (Alude, Cleary Chemical Corp., Dayton, NJ); a fosetyl Al fungicide formulated with a proprietary blue-green pigment (fosetyl Al/pigment) (Chipco Signature, Bayer Environmental Science, Montvale, NJ); a fosetyl Al fungicide with no pigment (fosetyl Al) (Aliette, Bayer Environmental Science, Montvale, NJ); a 1.0 M solution of reagent-grade phosphorous acid adjusted to a pH of 6.2 with 10.0 M potassium hydroxide (potassium phosphite); a solution of reagent-grade phosphoric acid adjusted to a pH of 6.2 with 10.0 M potassium hydroxide (potassium phosphate); a commercial formulation of mefenoxam (Subdue MAXX, Syngenta Crop Protection, Inc., Greensboro, NC); and an untreated control.

All phosphonate treatments were applied at equivalent amounts of phosphorous acid, based on the phosphorous acid equivalent listed on the Alude label and according to the molecular formula (Cohen and Coffey, 1986) and amount of fosetyl Al listed on the Aliette and Chipco Signature labels. The rate of phosphorous acid used for all phosphonate treatments in this study was 9.6 kg ha$^{-1}$, and was based on the intermediate product rate (23.6 L ha$^{-1}$) listed on the Alude label for Pythium diseases. Potassium phosphate was applied at 9.6 kg ha$^{-1}$ phosphoric acid and mefenoxam was applied at 0.76 kg ha$^{-1}$.

The experimental design was a split block with fungicide treatments serving as whole plots and grass species as sub plots. Each treatment was replicated four times. The whole plots were 0.91 by 2.44 m and sub plots were 0.91 by 1.22 m.

Five days prior to inoculation and 3 days prior to treatment application in 2004 and 2005, thiophanate methyl (3336 F, Cleary Chemical Corp., Dayton, NJ) was applied.
at 9.15 kg ha\(^{-1}\) to prevent brown patch and dollar spot caused by *Rhizoctonia solani* (Kuhn) and *Sclerotinia homoeocarpa* (F.T. Bennett), respectively.

Treatments were applied on 30 August, 2004 and 18 July, 2005 with a CO\(_2\)-powered backpack sprayer equipped with a single boom and 11008E nozzle. Applications were made at 275 kPa with a dilution rate equivalent to 814.8 L ha\(^{-1}\) H\(_2\)O. On 31 August, 2004 and 19 July, 2005, the open ends of the chamber were sealed with the plastic-covered end frames.

Turf encompassing the entire 132.9 m\(^2\) test area was inoculated on 1 September, 2004 and 20 July, 2005 with 34.1 L of a mycelia and rye (*Secale cereal* L.) grain slurry made from a five-isolate pool of *P. aphanidermatum* (P-3, P-20, P-38, P-40, and P-41). The inoculum was prepared by twice-autoclaving a mixture of rye grains and water, inoculating the mixture with the *P. aphanidermatum* isolates, and allowing the mixture to become fully colonized on a laboratory bench for 5 to 7 days. Just prior to inoculation, the colonized rye grain was placed in a blender with distilled water and ground into thick slurry (500 ml of colonized rye grain made approximately 2.7 L of slurry). The slurry was distributed over the test area by hand using a jar with a perforated lid. To insure uniform coverage, four passes were made over the entire test area in different directions. Immediately before inoculation, the misting system was activated for approximately 5 min and the chamber was sealed to maintain high temperatures and humidity. The misting system was activated periodically during the test period to cool turf and increase humidity. Test plots were not mowed between the day of treatment application and disease assessment (11-12 days after treatment).
Visual disease assessments were made on both grass species on 10 September, 2004 (9 days after inoculation and 11 days after treatments were applied) and 29 July, 2005 (9 days after inoculation and 11 days after treatments were applied). Assessments were based on the percentage of plot area showing Pythium blight symptoms (percentage of blighted turf), with 100 percent indicating completely blighted turf, and 0 percent equal to no visible blighted turf. Generally, blighted turf was severely damaged and did not recover. Data were subjected to analysis of variance and means were separated using Fisher’s Protected Least Significant Difference Test at $P = 0.05$.

**Results and Discussion**

*In vitro experiments with phosphorous acid, phosphoric acid, and mefenoxam*

Phosphorous acid EC$_{50}$ values for 23 isolates representing seven *Pythium* spp. ranged from 35.6 to 220.8 µg ml$^{-1}$ (Table 2). Phosphorous acid EC$_{50}$ values for the eight isolates of *P. aphanidermatum* ranged from 35.6 to 171.8 µg ml$^{-1}$, with the only mefenoxam-resistant isolate (Zen 97-375) showing the lowest EC$_{50}$ value. *Pythium aphanidermatum* isolates were more sensitive to mefenoxam than phosphorous acid, with mefenoxam EC$_{50}$ values for sensitive isolates ranging from 0.46 to 0.94 µg ml$^{-1}$. Phosphorous acid sensitivity of the other *Pythium* spp. fell within the range of *P. aphanidermatum* isolates, except for the isolate of *P. volutum* (EC$_{50}$ = 185.4 µg ml$^{-1}$) and one isolate of *P. myrotylium* (EC$_{50}$ = 220.8 µg ml$^{-1}$) (Table 2). No significant inhibition or stimulation of mycelial growth occurred for any *Pythium* isolate exposed to phosphoric acid (data not presented).
Although the degree of sensitivity of *Pythium* isolates to phosphonate fungicides was not determined *in vivo*, blighting caused by *P. aphanidermatum* isolates used to inoculate the field study (EC$_{50}$ values ranging from 94.6 to 134.3 µg ml$^{-1}$) was suppressed with phosphonate fungicides at rates listed on the fungicide labels. This is in agreement with a report by Sanders et al. (1990) in which blighting of creeping bentgrass caused by a *P. aphanidermatum* isolate with an EC$_{50}$ value of 275 µg ml$^{-1}$ was suppressed using 2.4 g m$^{-1}$ of Aliette 80W. Presumably, pathogenicity of all *P. aphanidermatum* isolates within the range of EC$_{50}$ values found in our study can be suppressed with label-prescribed rates of phosphonate fungicides.

To date, no field-resistance or reduced sensitivity of *Pythium* spp. to phosphonate fungicides has been reported. However, three *P. aphanidermatum* isolates exposed to a chemical mutagen had ED$_{50}$ values ranging from 3,000 to 4,700 µg ml$^{-1}$, and two of the isolates were completely insensitive to the Aliette 80W applied to creeping bentgrass at 2.4 and 3.7 g/m$^2$ in pot studies (Sanders et al., 1990). Two of the *P. aphanidermatum* isolates used in our *in vitro* study (PA 20 and PA 27) were isolated before phosphonate fungicides were used on golf courses; thus, the EC$_{50}$ values of 134.3 and 171.8 µg ml$^{-1}$ for these isolates serve as baseline values from which to compare *P. aphanidermatum* isolates where reduced sensitivity is suspected. We found no evidence for making assumptions on reduced sensitivity for any of the other *Pythium* spp. used in this study.

Few studies have been conducted to determine if differences in sensitivity to phosphonate fungicides occur among various *Pythium* spp. Fenn and Coffey (1984) reported significant differences in growth among four *Pythium* spp. in response to phosphorous acid on CMA. However, the authors used only one isolate to represent each
Table 2. Phosphorous acid EC$_{50}$ values for seven species and 23 isolates of *Pythium*, and mefenoxam EC$_{50}$ values for seven *P. aphanidermatum* isolates.

<table>
<thead>
<tr>
<th><em>Pythium</em> species</th>
<th>Isolate</th>
<th>Phosphorous acid EC$_{50}$ (µg ml$^{-1}$)†</th>
<th>Mefenoxam EC$_{50}$ (µg ml$^{-1}$)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>P-27</td>
<td>171.8</td>
<td>0.75</td>
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<td><em>P. aphanidermatum</em></td>
<td>P-20</td>
<td>134.3</td>
<td>0.46</td>
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<td><em>P. aphanidermatum</em></td>
<td>P-38</td>
<td>122.0</td>
<td>0.94</td>
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<td><em>P. aphanidermatum</em></td>
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<td>100.7</td>
<td>0.78</td>
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<td>0.61</td>
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<td>&gt;100</td>
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<td>132.9</td>
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<td>72076-96</td>
<td>118.1</td>
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<td><em>P. irregulare</em></td>
<td>42130-97</td>
<td>103.2</td>
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<td><em>P. myriotylum</em></td>
<td>P-49</td>
<td>220.8</td>
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<td><em>P. volutum</em></td>
<td>PRD 48</td>
<td>185.4</td>
<td></td>
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<td><em>P. graminicola</em></td>
<td>UAA1</td>
<td>143.2</td>
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</tbody>
</table>

† EC$_{50}$ values (effective concentration that reduces mycelial growth by 50%) were obtained by regressing relative growth against the log$_{10}$ of phosphorous acid or mefenoxam concentration.
species and did not examine intra-species variability. Because only a limited number of isolates of individual *Pythium* spp. were used in our study, no meaningful conclusions can be drawn concerning sensitivity differences among species. However, in some cases, the range of EC$_{50}$ values for isolates of one species overlap with those for other species, indicating that intra-species variation should be considered if attempting to distinguish phosphorous acid sensitivity among *Pythium* spp.

Comparisons of phosphorous acid EC$_{50}$ values from different studies should be interpreted with caution due to differences in experimental methods. Studies using phosphonate fungicides and phosphorous acid have revealed that factors such as pH, active ingredient (potassium phosphite vs. fosetyl Al), and phosphate concentration in media can influence the growth response of certain fungi (Abu-Jawdah, 1983; Barchietto et al., 1989; Fenn and Coffey, 1984). In the present study, phosphorous acid was adjusted to a pH of 6.2 and amended into CMA so that we could attempt a reasonable comparison of our findings with studies using similar methods (Fenn and Coffey, 1984; Sanders et al., 1990). Results from our study would likely change if experiments were performed using media other than CMA or if the pH of the phosphorous acid solution was significantly different from 6.2.

**Suppression of Pythium blight symptoms with potassium phosphite, fosetyl Al, and mefenoxam.**

In 2004, a treatment by turfgrass species interaction for the percentage of blighted turf was significant ($P > 0.0001$) (Table 3). The interaction revealed a difference in the magnitude of percentage of blighted turf between perennial ryegrass and creeping
bentgrass, with less symptom expression and greater symptom suppression with phosphonate fungicides on creeping bentgrass (Fig. 1) than on perennial ryegrass (Fig. 2).

On creeping bentgrass, no differences in percentage of blighted turf were detected among any of the fungicides used in the 2004 test (Fig. 1). On perennial ryegrass, mefenoxam provided a lower percentage of blighted turf than the potassium phosphite treatment and the potassium phosphite-C treatment; but was not different from either fosetyl Al treatment (Fig. 2). All phosphonate treatments provided > 89 percent suppression of blighted turf on perennial ryegrass and creeping bentgrass relative to the untreated control, and no differences in percentage of blighted turf were detected among any of the phosphonate treatments on either turfgrass species in 2004. The potassium phosphate treatment did not differ from the untreated control in percentage of blighted turf on either species.

In 2005, a significant treatment by species interaction occurred for the percentage of blighted turf ($P = 0.0195$) (Table 3). As in 2004, the interaction indicated a difference in the magnitude of percentage of blighted turf between perennial ryegrass and creeping bentgrass. Greater disease symptom suppression with phosphonate fungicides occurred on creeping bentgrass (Fig. 3) than on perennial ryegrass (Fig. 4).

All phosphonate treatments and mefenoxam provided a lower percentage of blighted turf than the untreated control and potassium phosphate treatment on creeping bentgrass and perennial ryegrass. Although suppression of Pythium blight symptoms with the phosphonate treatments was not as pronounced in 2005 as in 2004, no differences in percentage of blighted turf occurred among these treatments. Differences in the degree of Pythium blight suppression between 2004 and 2005 may be explained by
higher temperatures inside of the chamber in 2005. In 2004, only 57 h with temperatures over 30°C and 59 h under 20°C were recorded inside of the chamber; whereas in 2005, 75 h over 30°C and only 42 h under 20°C were recorded in the chamber. On creeping bentgrass, the mefenoxam treatment showed reduced percentage of blighted turf compared to the potassium phosphite-C treatment, but was not different from the fosetyl Al, fosetyl Al/pigment, and the potassium phosphite treatments (Fig. 3). On perennial ryegrass, mefenoxam provided greater suppression of Pythium blight symptoms than all other treatments (Fig. 4).

Results of the 2004 and 2005 field studies using commercial phosphonate fungicides and a solution of reagent-grade phosphorous acid with no formulation enhancements at equivalent rates of phosphorous acid revealed no differences in percent blighting among phosphonate treatments in either year of the study, regardless of active ingredient, formulation, or turfgrass species. These results are similar to other studies in which no differences in the efficacy of Pythium blight control were found among commercial fungicides containing fosetyl Al or potassium phosphite as the active ingredient (Datnoff et al., 2004; Datnoff et al., 2005; Soika and Tredway, 2007b; Uddin et al., 2002). In studies where differences in Pythium blight efficacy were found among certain phosphonate products, the reason may have been due to products being applied at different rates of phosphorous acid (Uddin et al., 2003; Uddin et al., 2004).

The findings from our field study may not necessarily hold for other diseases or hosts. In a study on Persica indica L., buffered phosphorous acid provided better control of stem infection caused by Phytophthora citricola Sawada compared with fosetyl Al when both treatments were applied at equal concentrations of phosphorous acid (Fenn
Table 3. Analysis of variance for percentage of blighted turf as influenced by treatments and turfgrass species.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F value</th>
<th>P value</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication (R)</td>
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<td>2.69</td>
<td>0.0769</td>
<td>0.82</td>
<td>0.510</td>
</tr>
<tr>
<td>Species (S)</td>
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<td>41.11</td>
<td>0.0077</td>
<td>7.43</td>
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<tr>
<td>R x S</td>
<td>3</td>
<td>1.26</td>
<td>0.3164</td>
<td>1.95</td>
<td>0.1574</td>
</tr>
<tr>
<td>Treatment (T)</td>
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<td>115.39</td>
<td>&lt;0.0001</td>
<td>30.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T x S</td>
<td>6</td>
<td>9.61</td>
<td>&lt;0.0001</td>
<td>3.43</td>
<td>0.0195</td>
</tr>
</tbody>
</table>
Fig. 1. Effect of phosphonate fungicides on Pythium blight development of cv. Penncross creeping bentgrass in 2004, expressed as percentage of blighted turf. Potassium phosphite-C is a commercial formulation of potassium phosphite (Alude), fosetyl Al is a commercial formulation fosetyl Al (Aliette), and fosetyl Al/pigment is a commercial formulation of fosetyl Al and a proprietary blue-green pigment (Chipco Signature). Bars with the same letter are not significantly different at $P = 0.05$. 
Fig. 2. Effect of phosphonate fungicides on Pythium blight development on cv. Integra perennial ryegrass in 2004, expressed as percentage of blighted turf. Potassium phosphite-C is a commercial formulation of potassium phosphite (Alude), fosetyl Al is a commercial formulation fosetyl Al (Aliette), and fosetyl Al/pigment is a commercial formulation of fosetyl Al and a proprietary blue-green pigment (Chipco Signature). Bars with the same letter are not significantly different at $P = 0.05$. 
Fig. 3. Effect of phosphonate fungicides on Pythium blight development of cv. Penncross creeping bentgrass in 2005, expressed as percentage of blighted turf. Potassium phosphite-C is a commercial formulation of potassium phosphite (Alude), fosetyl Al is a commercial formulation fosetyl Al (Aliette), and fosetyl Al/pigment is a commercial formulation of fosetyl Al and a proprietary blue-green pigment (Chipco Signature). Bars with the same letter are not significantly different at $P = 0.05$. 
Fig. 4. Effect of phosphonate fungicides on Pythium blight development on cv. Integra perennial ryegrass in 2005, expressed as percentage of blighted turf. Potassium phosphite-C is a commercial formulation of potassium phosphite (Alude), fosetyl Al is a commercial formulation fosetyl Al (Aliette), and fosetyl Al/pigment is a commercial formulation of fosetyl Al and a proprietary blue-green pigment (Chipco Signature). Bars with the same letter are not significantly different at $P = 0.05$. 
Cook et al. (2006) found that fosetyl Al formulated with a proprietary blue-green pigment (Chipco Signature) provided better suppression of anthracnose basal rot in putting greens compared with other phosphonate fungicides applied at equivalent rates of phosphorous acid. The fact that this formulation suppressed anthracnose symptoms and another fosetyl Al product did not, indicates that formulation differences may account for the improved anthracnose control. The blue-green pigment associated with the fosetyl Al may have an indirect beneficial effect on anthracnose suppression, perhaps by enhancing disease resistance mechanisms or shielding turfgrasses from debilitating environmental stresses which may predispose turfgrass to anthracnose (Vincelli and Dixon, 2005; Burpee, 2005).

In conclusion, results of this study demonstrate there is little difference between potassium phosphite and fosetyl Al fungicides with respect to suppression of Pythium blight when applied prior to symptom development at similar rates of phosphorous acid. However, the degree of Pythium blight suppression with phosphonate fungicides may be greater on creeping bentgrass than on perennial ryegrass. Practitioners should be able to successfully use either type of active ingredient preventatively in their Pythium blight management programs on perennial ryegrass and creeping bentgrass. Although phosphorous acid inhibited all Pythium spp. examined in the in vitro study, little information exists on suppression of Pythium diseases other than Pythium blight. More research is needed on the efficacy of phosphonate fungicides on Pythium root rot and dysfunction of turfgrasses, and how the respective pathogens are influenced by phosphorous acid.
Chapter 3

SUPPRESSION OF ANTHRACNOSE BASAL ROT AND IMPROVED PUTTING GREEN QUALITY WITH PHOSPHONATE FUNGICIDES

Introduction

Anthracnose basal rot, caused by *Colletotrichum cereale* Manns (Crouch, Clarke and Hillman) is a destructive disease of turfgrasses in many areas of the United States. In northern climates, this disease occurs on annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.) putting greens throughout the growing season, but is particularly severe during periods of high temperature and humidity (Smiley et al., 2005).

Golf course superintendents manage anthracnose basal rot using cultural practices and applications of fungicides when conditions are conducive for disease development (Vargas, 1994). Fungicides, including chlorothalonil, thiophanate-methyl, azoxystrobin, triadimefon, and propiconazole, are effective in controlling anthracnose, although efficacy varies depending on location, rate and timing of application, and other factors (Danneberger et al., 1983; Vargas, 1994; McCullough and Wong, 2005; Soika and Tredway, 2007a; Kaminski and Keneally, 2006).

Phosphonate fungicides are composed of the salts or esters of phosphorous acid, and most have potassium phosphite or fosetyl Al as active ingredients (Guest and Grant,
Phosphonate fungicides control many diseases caused by *Phytophthora* spp., *Pythium* spp., and other members of the Peronosporales order. These fungicides also suppress or control some diseases caused by pathogens unrelated to the Peronosporales, including nematode disorders caused by *Heterodera avenae* Woll. and *Meloidogyne marylandi* Jepson and Golden (Oka et al., 2007), bacterial wilt caused by *Ralstonia solanacearum* Smith Yabuuchi et al. (Norman et al., 2006), moldy-core decay caused by *Alternaria alternate* (Fr.) Keissl. (Reuveni et al., 2003), and in some cases, anthracnose basal rot (Burpee, 2005; Towers et al., 2003; Tredway, 2006). Often, phosphonate fungicides are not particularly effective in controlling anthracnose basal rot alone, and are combined with other fungicides that have a stronger fungitoxic affect on the pathogen (Burpee, 2005; Wong et al., 2007).

Although extensive research has been conducted on the fungitoxic effects of phosphonate fungicides on *Phytophthora* spp. and *Pythium* spp., relatively few *in vitro* studies have been carried out with *Colletotrichum* spp. (Fenn and Coffey, 1984; Abu-Jawdah, 1981; Burpee, 2005). Abu-Jawdah (1981) reported that spore germination of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. was reduced following a 36-h incubation period on solid media amended with 400 µg ml\(^{-1}\) of fosetyl Al as Aliette fungicide. Burpee (2005) reported that commercial formulations of two phosphonate fungicides, potassium phosphite and fosetyl Al, caused 50% inhibition (EC\(_{50}\)) of mycelial growth of an isolate of *C. graminicola* (presumably *C. cereale*) at concentrations of 121.9 and 364.6 mg ml\(^{-1}\), respectively. Burpee (2005) concluded that isolates of *C. graminicola* and *P. aphanidermatum* are significantly more sensitive to potassium phosphite and fosetyl Al *in vitro* than isolates of other common turfgrass pathogens (*Rhizoctonia solani*...
Field studies comparing phosphonate fungicides for anthracnose control have been conducted at different locations over multiple years, and using different formulations, rates, and application timings. In some of these studies, fosetyl Al and potassium phosphite products reduced anthracnose basal rot symptom development (Towers et al., 2003; Uddin and Tornquist, 2007; Wong et al., 2007; Tredway, 2006); whereas other studies showed no symptom suppression when these products were used alone (Green, 2001; Fidanza and Wilchak, 2004). In cases where symptom suppression occurred, the results may be influenced by product formulation and rate. Often, phosphonate products are not compared at equivalent concentrations of phosphorous acid, thus it is difficult to assess performance based on the active ingredient of the different products. Because of potential efficacy and price differences among the active ingredients and formulations of phosphonate fungicides, golf turf managers may benefit from comparisons of different formulations of phosphonate products when applied at the same concentration of phosphorous acid.

Phosphonate fungicides can improve turfgrass quality, especially when used on bentgrass and annual bluegrass putting greens in summer (Vincelli and Dixon, 2005; Dorer, 1996; Tredway, 2006). Studies examining the effects of phosphonate fungicides on turfgrass quality are often conducted using products that are formulated with dyes or pigments, and at least some of the quality enhancement has been attributed to the formulation (Mudge, 1997), especially in the case of the fosetyl Al-containing product Chipco Signature (Vincelli and Dixon, 2005). Few studies examining the effects of
potassium phosphite or fosetyl Al independent of formulation have been conducted on
turfgrasses managed as putting greens (Dorer, 1996).

The objectives of this study were to assess the response of four isolates of C.
cereale to different concentrations of phosphorous acid in vitro, determine if active
ingredient and formulation of phosphonate fungicides provide varying degrees of control
of anthracnose basal rot in the field when applied at equivalent rates of phosphorous acid,
and to determine if active ingredient and formulation of phosphonate fungicides provide
different effects on turfgrass quality when applied at equivalent rates of phosphorous
acid.

**Materials and Methods**

**Isolation, identification, and maintenance of C. cereale isolates**

During 2003, samples of annual bluegrass exhibiting anthracnose basal rot
symptoms were collected from a park in Clifton Springs, NY; a research green located at
the Joseph Valentine Turfgrass Research Center in University Park, PA; and a golf course
in Boalsburg, PA. The park in NY is at least two miles from the nearest golf course and
has no history of phosphonate fertilizer or fungicide treatments. The research green in
University Park is in close proximity to areas that have been treated with phosphonate
fungicides, and the golf course putting greens in Boalsburg were treated with
phosphonate fungicides in 2003.

Infected crown tissues collected from all three sampling sites were surface
sterilized in 0.6% sodium hypochlorite, rinsed in sterile distilled water, and placed on
half-strength potato dextrose agar (PDA). Developing colonies, similar in appearance to known cultures of *C. cereale*, were transferred to fresh plates of PDA and incubated at 19°C. Isolates were identified as *C. cereale* based on morphological descriptions by Sutton (1966, 1980) and Crouch et al. (2006). *Colletotrichum cereale* isolates used in the *in vitro* experiments were CS-3 and CS-7 (obtained from the NY site); D-2 (from the Valentine Turfgrass Research Center); and Elks-2A (from the Boalsburg, PA golf course). Each isolate was derived from a single conidium.

Isolates were maintained in long term storage by placing a 20- by 10-mm strip of sterile, moist Whatman filter paper on top of Petri dishes containing PDA, placing colonized plugs of each isolate adjacent to the strip, and allowing the colony to cover the filter paper strip. When fully colonized, each strip was dried and stored in a desiccator for several months. Just prior to use in experiments, small sections of the colonized filter paper strips were placed on full-strength (17 g L\(^{-1}\)) corn meal agar (CMA) (Becton, Dickinson, and Co., Sparks, MD, 21153) and allowed to colonize the medium.

**Effect of phosphorous acid and phosphoric acid on *C. cereale* in vitro**

An experiment was designed to assess the effect of different concentrations of phosphorous acid and phosphoric acid (H\(_3\)PO\(_4\)) on mycelial growth of isolates CS-3, CS-7, D-2, and Elks 2A. The sensitivity of these isolates to other fungicides was not determined in this experiment. Phosphorous acid solutions were prepared by adjusting a 1M solution of phosphorous acid to a pH of 6.2 by titrating with 10M potassium hydroxide (KOH) (Coffey and Joseph, 1985). Phosphoric acid solutions were made by adjusting a 1M solution of phosphoric acid to a pH of 6.2 by titrating with 10M KOH.
These solutions were used to amend full-strength CMA with 1000, 500, 100, 50, and 10 µg ml\(^{-1}\) phosphorous acid and 1000, 500, 100, 50, and 10 µg ml\(^{-1}\) phosphoric acid. The phosphoric acid/KOH-amended media contained similar concentrations of phosphorus and KOH to the phosphorous acid/KOH-amended media, and was included as a control to attempt to account for any nutritional, pH, or osmotic effects on growth that may result from the phosphorus or KOH. Corn meal agar not amended with phosphorous acid/KOH or phosphoric acid/KOH, also served as a control.

All \textit{C. cereale} isolates were maintained on non-amended CMA at 25°C for 10 days prior to transferring. Agar plugs, 7 mm in diameter, were cut from actively-growing margins of each colony and transferred to the center of plastic 90-mm-diameter Petri dishes containing CMA amended with the phosphorous acid/KOH, phosphoric acid/KOH; or non-amended CMA. Two replicate dishes were used for each isolate. All treatments were randomized in two blocks and incubated in the dark at 25°C. Radial growth was determined by measuring colony radius at two points on each dish and recording the average value. \textit{Colletotrichum cereale} radial growth measurements were taken 7 d after transfer. The experiment was performed twice. Because results from the two experiments were not significantly different, data were pooled prior to determining percent radial growth relative to the non-amended control for individual isolates.

**Anthracnose basal rot symptom suppression and turfgrass quality**

This experiment was conducted on a research putting green at the Joseph Valentine Turfgrass Research Center, University Park, PA during 2004 and 2005. The putting green soil is a uniform sandy loam with a pH of 7.2, 154.6 kg ha\(^{-1}\) Mehlich-3 P,
0.07 cmol exchangeable K kg\(^{-1}\) of soil, and a CEC of 6.2 cmol\(_e\) kg\(^{-1}\) of soil. The turfgrass is an eight-yr-old mixed stand of ‘Providence’ creeping bentgrass (~70%) and annual bluegrass (~30%). The turf was mowed at 3.2 mm with a triplex greens mower six times wk\(^{-1}\) during the growing season. Clippings were collected in baskets and removed from the site. The test area was fertilized with 97.6 kg ha\(^{-1}\) N as isobutylidene diurea (IBDU) in Oct. 2003 and 2004, and 24.4 kg ha\(^{-1}\) N as IBDU in June 2005. Vinclozolin (Curalan 50EG, BASF Corp., Research Triangle Park, NC) was applied at 1.6 kg ai ha\(^{-1}\) to the test area to control dollar spot in Sep. 2004 (after the 2004 test was terminated) and pentachloronitrobenzene (Turficide 10% Granular, Crompton Crop Protection, Middlebury, CT) was applied in Nov. 2004 at 49.2 kg ai ha\(^{-1}\) to prevent snow mold diseases. No fungicides were applied to the test area during spring and summer of 2004 and 2005, other than those used as treatments in the tests.

Two sets of treatments were included in the 2004 test. One set included a commercial formulation of a potassium phosphite fungicide (potassium phosphite-C) (Alude, Cleary Chemical Corp., Dayton, NJ), a fosetyl-Al fungicide formulated with a proprietary blue-green pigment (fosetyl Al/pigment) (Chipco Signature, Bayer Environmental Science, Montvale, NJ); a fosetyl Al fungicide with no pigment (fosetyl Al) (Aliette, Bayer Environmental Science, Montvale, NJ); a 1.0 M solution of reagent-grade phosphorous acid adjusted to a pH of 6.2 with 10.0 M KOH (non-commercial potassium phosphite); a solution of reagent-grade phosphoric acid adjusted to a pH of 6.2 with 10.0 M KOH (K-phosphate); and an untreated control. The second set of treatments included each of the treatments in the first set combined with vinclozolin (Curalan 50EG), and a vinclozolin control. All vinclozolin treatments were applied at 1.6 kg ha\(^{-1}\)
Vinclozolin was combined with each phosphonate treatment in the second set of treatments to control dollar spot disease (phosphonate fungicides do not control dollar spot) because this disease will damage unprotected turf plots and compromise data collection. Also, vinclozolin has very little effect on anthracnose basal rot, and presumably would not greatly influence results of the test (B. Clarke, personal communication).

Dollar spot disease became evident during late June 2004 in the treatments that did not contain vinclozolin; thus vinclozolin (1.6 kg ha$^{-1}$) was added to these treatments beginning with the 2 July 2004 application and throughout the remainder of the test. Although this change did not affect anthracnose basal rot ratings (all disease severity data was collected before the 2 July application), it could have influenced quality data after 2 July.

The 2005 test site was placed immediately adjacent to the 2004 test site. All treatments applied in the 2004 test were applied in the 2005 test. In addition to these treatments, thiophanate-methyl (3336F, Cleary Chemical Corp, Dayton, NJ) was applied at 7.6 kg ha$^{-1}$ alone and in combination with all phosphonate treatments except fosetyl Al to determine if efficacy would be enhanced with the addition of a fungicide with known efficacy on anthracnose basal rot. Thiophanate-methyl has a history of effective control of anthracnose basal rot at the Joseph Valentine Turfgrass Research Center. Another set of treatments included all of the thiophanate-methyl treatments combined with vinclozolin (Table 4).

All phosphonate treatments in the 2004 and 2005 tests were applied at equivalent amounts of phosphorous acid based on the phosphorous acid equivalent listed on the
potassium phosphite-C product label, and according to the molecular weight of the phosphorous acid portion of the chemical formula of fosetyl Al and amount of fosetyl Al listed on the product labels. The rate of phosphorous acid used in this study was 9.6 kg ha\(^{-1}\), and was based on the phosphorous acid equivalent of an intermediate product rate (13.8 kg ha\(^{-1}\) fosetyl Al) listed on the fosetyl-Al/pigment product label for anthracnose diseases, and for summer stress complex on the potassium phosphite-C product label (14.8 kg ha\(^{-1}\) potassium phosphite). The rates of active ingredient for all phosphonate treatments, potassium phosphate, vinclozolin, and thiophanate-methyl are provided in Table 4.

The experimental design was a randomized complete block design with four replications. Plot size was 3.1 by 0.91 m. In 2004, all treatments were applied every 14 d beginning on 21 May and ending 13 Aug. for a total of seven applications. In 2005, all treatments were applied every 14 d beginning on 4 May and ending 29 July for a total of seven applications. Treatments were applied with a CO\(_2\)-powered backpack sprayer equipped with a single boom fitted with a 11008E nozzle. Applications were made at 275 kPa with a dilution rate equivalent to 814.8 L H\(_2\)O ha\(^{-1}\).

Anthracnose basal rot ratings were made when disease became severe enough to provide an adequate visual evaluation and was uniformly distributed over the entire test area. Turf quality ratings were made every 14 d, just prior to treatment applications. Disease severity was visually assessed on a scale of 0 to 10, with 10 indicating severe disease symptoms and 0 indicating no visible symptoms. Quality was assessed visually using a scale of 0 to 10, with 10 indicating excellent turf quality and 0 indicating extremely poor quality turf. Disease severity and quality data were subjected to analysis.
of variance and means were separated using Fisher’s Protected Least Significant Difference Test at the 0.05 level of significance.

**Results and Discussion**

**Effect of phosphorous acid and phosphoric acid on *C. cereale* in vitro**

None of the four *C. cereale* isolates were strongly inhibited at any of the concentrations of phosphorous or phosphoric acid used in this experiment. Therefore, EC$_{50}$ values were not calculated. Radial growth of isolates CS-3 and CS-7 actually increased as concentrations of phosphorous acid and phosphoric acid increased. The radial growth of CS-3 and CS-7 at 1000 µg ml$^{-1}$ phosphorous acid, relative to growth of these isolates on non-amended CMA, was 153% and 171%, respectively. Isolates CS-3 and CS-7 were also stimulated by phosphoric acid treatments, with relative growth of 153% and 194%, respectively, at 1000 µg ml$^{-1}$ phosphoric acid. The increase in radial growth of these isolates with increasing concentrations of phosphorous acid and phosphoric acid indicates that these compounds may be serving as nutrient sources, or that they are favorably altering some physical or chemical attribute of the medium. Adams and Conrad demonstrated that several soil bacteria could utilize phosphtite as a phosphorus source (1952).

Isolates D-2 and Elks-2A did not exhibit increased growth as concentrations of phosphorous acid increased. Relative growth of D-2 and Elks-2A at 1000 µg ml$^{-1}$ phosphorous acid was 96% and 93%, respectively. Growth of these isolates increased slightly relative to the controls at the highest concentration of phosphoric acid (1000 µg
ml$^{-1}$) with relative growth approaching 107% and 112% for D-2 and Elks-2A, respectively. While it is conceivable that phosphorous acid concentrations higher than 1000 µg ml$^{-1}$ would significantly inhibit some or all of the C. cereale isolates, they appear less sensitive to phosphorous acid than P. aphanidermatum. Using the same media and methods presented in the materials and methods, EC$_{50}$ values of eight P. aphanidermatum isolates ranged from 35.1 - 173.4 µg ml$^{-1}$ phosphorous acid (unpublished data).

Burpee (2005) found that commercial formulations of potassium phosphite and fosetyl Al caused 50% inhibition (EC$_{50}$) of mycelial growth of an isolate of C. graminicola (CG00-06) at concentrations of 121.9 and 364.6 mg ml$^{-1}$, respectively. The author reported that 11 other C. graminicola isolates were less sensitive than CG00-006, and suggested that sensitivity differences among isolates may explain some of the variability in efficacy reported in field trials. The results of this in vitro study appear to confirm previous observations of variable response to phosphonate compounds across isolates.

Burpee (2005) concluded that isolates of C. graminicola and P. aphanidermatum are significantly more sensitive to potassium phosphite and fosetyl Al in vitro than isolates of other common turfgrass pathogens, and that it is conceivable that field application rates could reach levels in planta that may suppress C. graminicola.

The results of our experiment are difficult to compare to the findings of Burpee (2005) because of the different methods and materials used in the experiments. Burpee (2005) used formulated products amended into PDA; whereas we used technical grade phosphorous acid, adjusted to a pH of 6.2 with KOH, in CMA. Previous in vitro studies
using phosphonate fungicides have shown that factors such as concentration of phosphate in media (Fenn and Coffey, 1984; Barchietto et al., 1989), pH of the medium (Abu-Jawdah 1983; Barchietto et al., 1989), and type of phosphonate compound used in the media (Fenn and Coffey, 1984) influence the response of certain fungi to phosphonate fungicides.

Additional studies of uptake, accumulation, and antifungal activity of phosphonate fungicides in turfgrass plants are needed before scientists can accurately assess the fungitoxic impact of phosphonate fungicides on *C. cereale*.

**Anthracnose basal rot symptom suppression and turfgrass quality**

*Anthracnose basal rot control, 2004 results:* Anthracnose basal rot symptoms were observed on annual bluegrass in late June of 2004. The only treatments that showed a significant reduction in disease severity compared to the untreated control and vinclozolin treatment were fosetyl Al/pigment and the fosetyl Al/pigment + vinclozolin (Table 4).

*Anthracnose basal rot control, 2005 results:* A severe infestation of anthracnose basal rot occurred on annual bluegrass in early July 2005, and the test was rated on 5 July. Of the phosphonate treatments with no vinclozolin or thiophanate-methyl added, only the fosetyl Al/pigment and the potassium phosphite treatments showed a reduction in anthracnose basal rot severity relative to the untreated control (Table 4). As in 2004, the fosetyl Al/pigment treatment provided significantly better suppression of disease symptoms than all other phosphonate treatments. The vinclozolin treatment did not
reduce the severity of anthracnose basal rot symptoms when compared to the untreated control. None of the phosphonate + vinclozolin treatment combinations reduced anthracnose basal rot symptom expression when compared to the corresponding phosphonate treatments.

The thiophanate-methyl treatment caused a reduction in disease severity compared to the untreated control, and the phosphonate + thiophanate-methyl treatment combinations provided lower disease severity ratings than the thiophanate-methyl treatment. The thiophanate-methyl + potassium phosphite-C treatment provided better suppression of anthracnose basal rot symptoms than the potassium phosphite-C treatment and the thiophanate-methyl treatment.

Three-way combinations of phosphonates + vinclozolin + thiophanate-methyl did not perform better with respect to anthracnose basal rot symptom suppression than any of the phosphonate + vinclozolin or phosphonate + thiophanate-methyl treatments. All treatment combinations containing fosetyl Al/pigment (fosetyl Al/pigment + vinclozolin, fosetyl Al/pigment + thiophanate-methyl, and fosetyl Al/pigment + vinclozolin + thiophanate-methyl) provided better suppression of anthracnose basal rot symptoms than all other treatment combinations. However, none of these fosetyl Al/pigment combination treatments provided better symptom suppression than fosetyl Al/pigment alone.

_Turfgrass quality results for 2004:_ Turf quality data in 2004 revealed differences among treatments 14 d following the first application and on all subsequent rating dates (Table 5). Phosphonate treatments provided better quality than the untreated control on
Table 4. Treatments, rates, and anthracnose basal rot disease severity ratings for 2004 and 2005 phosphonate fungicide tests.

<table>
<thead>
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<th>Treatment†</th>
<th>Rate‡ (kg ai ha⁻¹)</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 June</td>
<td>5 July</td>
</tr>
<tr>
<td>Control</td>
<td>----</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>1.6</td>
<td>2.5 a</td>
<td>5.5 ab</td>
</tr>
<tr>
<td>K-phosphate</td>
<td>25.5</td>
<td>2.8 a</td>
<td>6.3 a</td>
</tr>
<tr>
<td>NC K-phosphite</td>
<td>21.7</td>
<td>2.0 ab</td>
<td>3.5 def</td>
</tr>
<tr>
<td>C K-phosphite</td>
<td>14.8</td>
<td>2.0 ab</td>
<td>4.5 bcd</td>
</tr>
<tr>
<td>Fosetyl-Al</td>
<td>13.8</td>
<td>2.5 a</td>
<td>4.5 bcd</td>
</tr>
<tr>
<td>Fosetyl-Al/pigment</td>
<td>13.8</td>
<td>1.0 c</td>
<td>2.0 gh</td>
</tr>
<tr>
<td>K-phosphate + vinclozolin</td>
<td>25.5 + 1.6</td>
<td>2.5 a</td>
<td>5.0 bc</td>
</tr>
<tr>
<td>NC K-phosphite + vinclozolin</td>
<td>21.7 + 1.6</td>
<td>2.0 ab</td>
<td>3.5 def</td>
</tr>
<tr>
<td>C K-phosphite + vinclozolin</td>
<td>14.3 + 1.6</td>
<td>1.8 ab</td>
<td>3.5 def</td>
</tr>
<tr>
<td>Fosetyl-Al + vinclozolin</td>
<td>13.8 + 1.6</td>
<td>1.8 ab</td>
<td>3.5 def</td>
</tr>
<tr>
<td>Fosetyl-Al/pigment + vinclozolin</td>
<td>13.8 + 1.6</td>
<td>0.5 c</td>
<td>1.3 h</td>
</tr>
<tr>
<td>T-methyl</td>
<td>7.6</td>
<td>---</td>
<td>4.0 cde</td>
</tr>
<tr>
<td>T-methyl + NC K-phosphite</td>
<td>7.6 + 21.7</td>
<td>---</td>
<td>2.8 fg</td>
</tr>
<tr>
<td>T-methyl + C K-phosphite</td>
<td>7.6 + 14.8</td>
<td>---</td>
<td>2.8 fg</td>
</tr>
<tr>
<td>T-methyl + fosetyl-Al/pigment</td>
<td>7.6 + 13.8</td>
<td>---</td>
<td>1.3 h</td>
</tr>
<tr>
<td>T-methyl + vinclozolin</td>
<td>7.6 + 1.6</td>
<td>---</td>
<td>3.5 def</td>
</tr>
<tr>
<td>T-methyl + vinclozolin + NC K-phosphite</td>
<td>7.6+1.6+43.6</td>
<td>---</td>
<td>3.0 efg</td>
</tr>
<tr>
<td>T-methyl + vinclozolin + C K-phosphite</td>
<td>7.6+1.6+14.8</td>
<td>---</td>
<td>2.8 fg</td>
</tr>
<tr>
<td>T-methyl + vinclozolin + fosetyl-Al/pigment</td>
<td>7.6+1.6+13.8</td>
<td>---</td>
<td>1.3 h</td>
</tr>
</tbody>
</table>

† Vinclozolin = Curalan 50EG; K-phosphate = reagent grade H₃PO₄/KOH; NC K-phosphite = non-commercial K-phosphite made with H₃PO₄/KOH; C K-phosphite = Alude, a commercial formulation of K-phosphite; fosetyl-Al = Aliette, a commercial formulation of fosetyl-Al; fosetyl-Al/pigment = Chipco Signature, a commercial formulation of fosetyl-Al formulated with a proprietary blue-green pigment; t-methyl = 3336F, a commercial formulation of thiophanate-methyl.
‡ All treatments containing K-phosphite or fosetyl-Al were applied at rates equivalent to 9.6 kg ha⁻¹ phosphorous acid.
§ Anthracnose basal rot disease severity ratings based on a 0-10 scale, 0 = no disease and 10 = severe disease symptoms.
¶ Data means within the same column and followed by the same letter are not significantly different as determined by Fisher’s Protected Least Significant Difference test at \( P=0.05 \).
most rating dates. The fact that vinclozolin was added to these treatments, including the untreated control, beginning on 2 July, 2004 and throughout the remainder of the 2004 test period, may have influenced turf quality. Although some differences in turfgrass quality were noted among the fosetyl Al, potassium phosphite-C, and the potassium phosphite treatments, numerical quality values were usually within a single whole unit, indicating that these differences were subtle. These results indicate that potassium phosphite and fosetyl Al have similar effects on turf quality when applied at equivalent concentrations of phosphorous acid. The fosetyl Al/pigment treatment produced higher quality ratings than the other phosphonate treatments on three rating dates. Two of these dates were in late June and early July, around the time that anthracnose began to appear in the test area. It is possible that the improved anthracnose suppression with fosetyl Al/pigment resulted in improved turf quality on these rating dates. The vinclozolin treatment produced quality ratings similar to the phosphonate treatments in June. The vinclozolin + phosphonate combination treatments generally produced similar or better turf quality than phosphonate treatments.

**Turfgrass quality results 2005:** Turf quality data in 2005 revealed differences among treatments 14 d following the first application and on all subsequent rating dates (Table 6). Differences between the untreated control and the fosetyl Al, potassium phosphite-C, and the potassium phosphite treatments were not apparent early in the test period, but became pronounced as the season progressed and the number of applications increased. Few differences were noted among the fosetyl Al, potassium phosphite-C, and the potassium phosphite treatments during 2005. However, the fosetyl-Al/pigment
Table 5. Treatments, rates, and quality ratings for the 2004 phosphonate fungicide test.

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Rate‡ (kg ai ha⁻¹)</th>
<th>Turf Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May 21</td>
<td>June 4</td>
</tr>
<tr>
<td>Control</td>
<td>----</td>
<td>5.8 a</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>1.6</td>
<td>5.8 a</td>
</tr>
<tr>
<td>K-phosphate</td>
<td>25.5</td>
<td>5.5 a</td>
</tr>
<tr>
<td>NC K-phosphate</td>
<td>21.7</td>
<td>6.0 a</td>
</tr>
<tr>
<td>C K-phosphate§</td>
<td>14.8</td>
<td>5.8 a</td>
</tr>
<tr>
<td>Fosetyl-Al¶</td>
<td>13.8</td>
<td>6.0 a</td>
</tr>
<tr>
<td>K-phosphate /pigment¶</td>
<td>13.8</td>
<td>5.8 a</td>
</tr>
<tr>
<td>+ vinclozolin</td>
<td>25.5+1.6</td>
<td>5.5 a</td>
</tr>
<tr>
<td>NC K-phosphate + vinclozolin</td>
<td>21.7+1.6</td>
<td>5.5 a</td>
</tr>
<tr>
<td>C K-phosphate + vinclozolin</td>
<td>14.8+1.6</td>
<td>5.5 a</td>
</tr>
<tr>
<td>Fosetyl-Al + vinclozolin</td>
<td>13.8+1.6</td>
<td>5.5 a</td>
</tr>
<tr>
<td>Fosetyl-Al /pigment + vinclozolin</td>
<td>13.8+1.6</td>
<td>5.8 a</td>
</tr>
</tbody>
</table>

† Vinclozolin = Curalan 50EG; K-phosphate = reagent grade H₃PO₄/KOH; NC K-phosphate = non-commercial K-phosphate made with H₃PO₄/KOH; C K-phosphate = Alude, a commercial formulation of K-phosphate; fosetyl-Al = Aliette, a commercial formulation of fosetyl-Al; fosetyl-Al/pigment = Chipco Signature, a commercial formulation of fosetyl-Al formulated with a proprietary blue-green pigment.
‡ All treatments containing K-phosphate or fosetyl-Al were applied at rates equivalent to 9.6 kg ha⁻¹ phosphorous acid.
§ Turf quality ratings based on a 0-10 scale, 10 = excellent turf quality 0 = poor turf quality.
¶ Dollar spot disease became evident during late June in treatments that did not contain vinclozolin, thus vinclozolin was added to these treatments beginning with the 2 July application and throughout the remainder of the test.
# Data means within the same column and followed by the same letter are not significantly different as determined by Fisher’s Protected Least Significant Difference test at P=0.05.
treatment produced higher quality ratings than the other phosphonate treatments on five out of eight rating dates. The vinclozolin + phosphonate and thiophanate-methyl + phosphonate combination treatments generally produced turf quality similar to the phosphonate treatments, thus, there did not seem to be a significant advantage with respect to quality improvement by combining phosphonates with other fungicides.

Of the phosphonate fungicide treatments included in this study, the fosetyl Al/pigment generally provided the most consistent and highest degree of anthracnose basal rot suppression, and the highest quality ratings over two growing seasons. Although the blue-green pigment in the fosetyl Al/pigment treatment can mask yellow and brown colors in turf foliage, any masking effect in this study appeared to be minimal due to the fact that quality ratings were taken two weeks after treatment application and daily mowing removed most leaf tissues containing pigment. Fewer brown patches, thicker turf, and more uniform turf were noted in fosetyl Al/pigment-treated plots than in other treatments on several rating dates. Similar results have been reported in other field studies using fosetyl Al/pigment in various regions of the United States (Uddin et al., 2004; Towers et al., 2003; Tredway, 2006). The fact that fosetyl Al/pigment and fosetyl Al treatments contained the same amount and type of active ingredient indicates that formulation may account for the improved anthracnose control and turfgrass quality with fosetyl Al/pigment.

The blue-green pigment (and perhaps other proprietary formulation enhancements) associated with the fosetyl Al/pigment formulation, but not the fosetyl Al formulation, has been suggested as a possible reason for improved disease control,
Table 6. Treatments, rates, and quality ratings for the 2005 phosphonate fungicide test.

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Rate‡ (Kg ai ha⁻¹)</th>
<th>May 4</th>
<th>May 18</th>
<th>June 1</th>
<th>June 15</th>
<th>June 29</th>
<th>July 13</th>
<th>July 27</th>
<th>Aug 10</th>
<th>(0-10) §</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>----</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.3 de</td>
<td>4.5 fg</td>
<td>5.3 e</td>
<td>4.5 j</td>
<td>4.5 d</td>
<td>4.3 g</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Vinclozalin</td>
<td>6.0 a</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.3 de</td>
<td>4.5 fg</td>
<td>5.3 e</td>
<td>4.5 j</td>
<td>4.5 d</td>
<td>4.3 g</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>K-phosphate</td>
<td>25.5</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.0 e</td>
<td>4.8 efg</td>
<td>5.8 cd</td>
<td>4.3 j</td>
<td>5.0 d</td>
<td>4.0 g</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>NC K-phosphate</td>
<td>21.7</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.3 de</td>
<td>5.0 def</td>
<td>5.8 cd</td>
<td>6.0 bc</td>
<td>6.8 cde</td>
<td>6.5 cde</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>C K-phosphate</td>
<td>14.8</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.8 bc</td>
<td>4.5 fg</td>
<td>6.0 bc</td>
<td>5.5 gh</td>
<td>6.5 c</td>
<td>5.8 f</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Fosetyl-Al</td>
<td>13.8</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.5 cd</td>
<td>4.8 efg</td>
<td>5.5 de</td>
<td>6.8 cde</td>
<td>6.5 c</td>
<td>6.5 cde</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Fosetyl-Al/pigment</td>
<td>13.8</td>
<td>6.0 a</td>
<td>7.0 a</td>
<td>6.8 a</td>
<td>6.0 ab</td>
<td>7.0 a</td>
<td>7.5 ab</td>
<td>7.3 abc</td>
<td>7.3 abc</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>K-phosphate + vinclozalin</td>
<td>25.5 + 1.6</td>
<td>6.0 a</td>
<td>6.3 bc</td>
<td>5.3 de</td>
<td>5.0 def</td>
<td>6.0 bc</td>
<td>4.8 ij</td>
<td>5.3 d</td>
<td>4.0 g</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>NC K-phosphate + vinclozalin</td>
<td>21.7 + 1.6</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.5 cd</td>
<td>5.0 def</td>
<td>6.0 bc</td>
<td>6.5 def</td>
<td>6.8 bc</td>
<td>6.0 ef</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>C K-phosphate + vinclozalin</td>
<td>14.8 + 1.6</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>6.0 b</td>
<td>5.5 bcd</td>
<td>6.0 bc</td>
<td>6.8 cde</td>
<td>6.8 bc</td>
<td>6.0 ef</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Fosetyl-Al + vinclozalin</td>
<td>13.8 + 1.6</td>
<td>6.0 a</td>
<td>6.3 bc</td>
<td>6.0 b</td>
<td>4.8 efg</td>
<td>5.8 cd</td>
<td>6.3 ef</td>
<td>6.8 bc</td>
<td>6.3 def</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Fosetyl-Al/pigment + vinclozalin</td>
<td>13.8 + 1.6</td>
<td>6.0 a</td>
<td>7.0 a</td>
<td>7.0 a</td>
<td>6.0 ab</td>
<td>7.0 a</td>
<td>6.8 cde</td>
<td>7.3 abc</td>
<td>7.5 ab</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl</td>
<td>7.6</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.8 bc</td>
<td>4.3 g</td>
<td>6.3 b</td>
<td>6.5 def</td>
<td>7.0 bc</td>
<td>5.8 f</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + NC K-phosphate</td>
<td>7.6 + 21.7</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>5.8 bc</td>
<td>5.3 cde</td>
<td>6.3 b</td>
<td>7.5 ab</td>
<td>7.3 abc</td>
<td>6.8 bcde</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + C K-phosphate</td>
<td>7.6 + 14.8</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>6.0 b</td>
<td>4.3 g</td>
<td>6.0 bc</td>
<td>7.0 bcd</td>
<td>7.5 ab</td>
<td>6.8 bcde</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + fosetyl-Al/pigment</td>
<td>7.6 + 13.8</td>
<td>6.0 a</td>
<td>7.0 a</td>
<td>7.0 a</td>
<td>5.8 bc</td>
<td>7.0 a</td>
<td>7.5 ab</td>
<td>8.0 a</td>
<td>8.0 a</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + vinclozalin</td>
<td>7.6 + 1.6</td>
<td>6.0 a</td>
<td>6.0 c</td>
<td>6.0 b</td>
<td>4.5 fg</td>
<td>6.0 bc</td>
<td>6.0 fg</td>
<td>6.5 c</td>
<td>6.0 ef</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + NC K-phosphate + vinclozalin</td>
<td>7.6 + 21.7</td>
<td>6.0 a</td>
<td>6.3 bc</td>
<td>6.0 b</td>
<td>4.8 efg</td>
<td>6.0 bc</td>
<td>7.0 bcd</td>
<td>7.5 ab</td>
<td>7.0 bcd</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + C K-phosphate + vinclozalin</td>
<td>7.6 + 14.8</td>
<td>6.0 a</td>
<td>6.5 b</td>
<td>6.0 b</td>
<td>4.3 g</td>
<td>6.0 bc</td>
<td>7.3 bc</td>
<td>7.0 bc</td>
<td>7.0 bcd</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>T-methyl + vinclozalin + fosetyl-Al/pigment</td>
<td>7.6 + 1.6</td>
<td>6.0 a</td>
<td>7.0 a</td>
<td>7.0 a</td>
<td>6.5 a</td>
<td>7.0 a</td>
<td>8.0 a</td>
<td>8.0 a</td>
<td>8.0 a</td>
<td>4.0 - 7.0</td>
</tr>
</tbody>
</table>

†Vinclozalin = Curalan; K-phosphate = reagent grade H₃PO₄/KOH; NC K-phosphate = non-commercial K-phosphate; C K-phosphate = Alude, a commercial K-phosphate; fosetyl-Al = Aliette, a commercial fosetyl-Al; fosetyl-Al/pigment = Chipco Signature, a commercial fosetyl-Al formulated with a proprietary blue-green pigment; t-methyl = 3336F, a commercial thiophanate-methyl.
‡All treatments containing potassium phosphite or fosetyl aluminum were applied at rates equivalent to 9.6 kg ha⁻¹ phosphorous acid.
§Turf quality ratings based on a 0-10 scale, 10 = excellent turf quality 0 = poor turf quality.
¶Data means within the same column and followed by the same letter are not significantly different as determined by Fisher’s Protected Least Significant Difference test at P=0.05.
turfgrass quality, and cosmetic improvement (Vincelli and Dixon, 2005; Burpee, 2005). Burpee (2005) stated that reduced toxicity of fosetyl Al/pigment *in vitro* compared to fosetyl Al, indicates that the pigment in the formulation does not enhance, and may interfere with fungistasis of *C. graminicola*. These findings suggest that the fosetyl Al/pigment may have an indirect beneficial effect on anthracnose suppression, perhaps by enhancing disease resistance mechanisms in turfgrasses, or shielding turfgrasses from debilitating environmental stresses which may predispose plants to anthracnose. Recent studies in Virginia indicate label rates of fosetyl Al/pigment applied to creeping bentgrass enhance superoxide dismutase levels in tissue for as many as 20 days of supraoptimal temperatures. Superoxide dismutase is an important antioxidant enzyme that is correlated with improved tolerance to heat stress and a delay in leaf senescence in turfgrasses (Erik Ervin, personal communication).

The only other phosphonate treatment that provided some degree of anthracnose basal rot control relative to the untreated control was the potassium phosphite treatment, but this only occurred in 2005 and was less effective than fosetyl Al/pigment. Because no special formulation enhancements were used in the preparation of this treatment, the suppressive effect can be attributed solely to the phosphorous acid. When applied alone, the potassium phosphite-C formulation did not suppress anthracnose basal rot when compared to the untreated control; however, when it was applied with thiophanate-methyl, control was improved over both potassium phosphite-C and thiophanate-methyl alone.

Some researchers have published data showing significant anthracnose suppression with potassium phosphite fungicides (Uddin and Tornquist, 2007; Tredway,
2006; Kaminski and Keneally, 2006), whereas others report none to limited suppression (Soika and Tredway, 2007a; Burpee et al., 2005a). Such conflicting data may reflect differences in disease severity among studies, length of time between the initial preventative application and symptom expression, the number of fungicide applications prior to disease ratings, and possibly other factors. Poor suppression of anthracnose symptoms with potassium phosphite was reported in studies where abnormally high temperatures, heavy rainfall, or drought resulted in severe anthracnose. Poor suppression was also found where disease symptoms were rated soon after initial treatments were applied, or in cases where symptoms were present before treatments were applied (Soika and Tredway, 2007a; Burpee et al., 2005a). In studies where temperature and precipitation extremes were not reported, and where two or more applications of potassium phosphite were made prior to symptom expression, some suppression was noted (Uddin and Tornquist, 2007; Tredway, 2006; Kaminski and Keneally, 2006). Until the conditions required for consistent anthracnose suppression with phosphite fungicides are better understood, practitioners will likely experience variable results in anthracnose control programs.

Phosphonate treatments provided better turf quality than the untreated control on most rating dates over the two-year test period. Although some significant differences in turfgrass quality were noted among the fosetyl Al, potassium phosphite-C, and the potassium phosphite treatments in both years of the study, differences were generally subtle. These results indicate that potassium phosphite and fosetyl Al have similar effects on turf quality when applied at equivalent amounts of phosphorous acid. Currently, we are unsure why phosphonate fungicides improve turfgrass quality. Quality improvement
does not appear to be caused by a nutritional effect, but may be partially due to a reduction in minor root-debilitating pathogens in putting green turf (McDonald et al., 2001). More detailed research may reveal how phosphonate fungicides improve turf quality, and provide insight into the environmental and management conditions under which these observations are made.
LITERATURE CITED


